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Deuterium and living organisms

Robertson Pratt

The existence of more than one variety of water is of great interest to physiologists, and during the three years that deuterium oxide, or heavy water, has been available for research many investigators have studied its influence on plants and animals.

Soon after Urey and his associates (33, 34)[1] isolated deuterium, an isotope of hydrogen, methods were developed for the concentration of D_2O, or deuterium oxide, from water by means of electrolysis. Nearly all samples of water, whether from the ocean, fresh water lakes, or formed by the combustion of organic matter, contain about 1 part in 5000, or 0.02 per cent, D_2O by weight. To isolate the D_2O from water, use is made of the well known fact that when an electric current passes through water, oxygen and hydrogen are liberated at the electrodes.[2] At first relatively more light than heavy hydrogen is liberated. Consequently as the treatment proceeds the ratio of heavy to light hydrogen in the residue increases and ultimately oxygen and deuterium are liberated. The gases are then burned and just as two atoms of hydrogen (mass = 1) may combine with one atom of oxygen to form a molecule of H_2O, so two atoms of deuterium (mass = 2) combine with one of oxygen to form D_2O. The cost of the current consumed is the greatest item of expense in this procedure, but improved methods and increased efficiency have combined to reduce the cost of production from about one dollar to approximately ten cents a drop.

Influence of D_2O on growth.—High concentrations of D_2O have been found in a number of cases to exert a depressing action on the normal growth of plants and animals. Tobacco seeds were found to sprout slowly in 50 per cent D_2O and not at all in 100 per cent D_2O (14, 15). Seeds were not killed, how-

[1] Numbers in parentheses refer to literature citations.

[2] In practice a dilute sodium hydroxide solution is prepared with the water to be concentrated. This facilitates electrolysis (4).

ever, for upon removal after three weeks to ordinary water they sprouted, although their development was somewhat abnormal. In other studies the rate of development of frogs' eggs decreased as the concentration of D_2O increased from 5 per cent to 30 per cent (35) and the development of fertilized sea urchins' eggs was promptly arrested when they were placed in contact with 99.5 per cent D_2O (17).

Neither bacteria nor lower plants are immune to the action of D_2O. Species of luminous bacteria and of *Euglena* failed to grow in concentrated heavy water, but it was observed that transfer to ordinary water after forty-one and nine days, respectively, resulted in renewal of normal growth (11). Other investigators found that different species of unicellular green algae did not grow if the D_2O content of the nutrient solution exceeded 85 per cent, although cells survived exposure to high concentrations for several days and resumed growth when returned to culture media with low concentrations (25). Fungus mycelia behaved similarly (13). The spores of the fungus that produces the powdery mildew disease of wheat also proved to be useful in a detailed study of the influence of D_2O on growth (22a). Elongation of the germ tubes was studied in concentrations ranging from 0.02 to 100 per cent. It was found that D_2O reduced the maximum rate and final amount of growth, that elongation of germ-tubes was promptly arrested when spores were placed in 100 per cent D_2O, and that spores were more sensitive to the deleterious action of D_2O after growth had begun in H_2O. Practically no growth occurred when the D_2O concentration was greater than 75 per cent.

Within certain limits of time, spores were not killed by exposure to 100 per cent D_2O, since upon return to H_2O they resumed growth. It was clear, however, that the injury resulting from prolonged exposure was, at least in part, irreversible. With exposures ranging from 24 to 124 hours, the length which germ tubes attained and the duration of the growth period in H_2O were inversely related to the previous period of immersion in 100 per cent D_2O.

Since high concentrations of D_2O exert a marked depressing action on normal growth, several investigators have tested this agent as a possible tool to be used in the treatment of cancers and other abnormal growths. Their efforts have all been unsuccessful. Under the conditions of the experiments, rodent tumors

and cancers have outwardly been entirely unaffected by concentrated or dilute D_2O (23, 29, 30, 37).

One experimenter failed to note any significant effect of 94 per cent D_2O on cell division of germinating wheat embryos (19) and another reported that the growth of *Staphylococcus* sp. and the typhus bacillus was unaffected over a period of 70 days by 92–94 per cent D_2O (10).

Stimulation of organisms exposed to dilute D_2O—i.e., concentrations less than 1 per cent—has been reported. Improved growth of various species of protozoa, bacteria, algae, fungi, and green plants has been said to result from treatment with 0.05–0.13 per cent D_2O (1, 9, 20, 26). Slight inhibition of the germination of *Lupinus* seeds and growth of the seedlings has also been reported (18).

Other workers, however, have been unable to detect any influence of carefully purified dilute heavy water on living organisms. In a number of experiments cultures of several species of protozoa, luminous bacteria, soil bacteria, and different pathogenic organisms (in vivo and in vitro) were not influenced by dilute D_2O (5, 11, 27). In other tests, fungi and the roots of wheat plants grew equally well in 0.46 per cent D_2O and in H_2O (7, 22).

Influence of D_2O on photosynthesis.—A study of the influence of D_2O on photosynthesis is of particular interest, since water enters directly into the chemical reactions at one stage of the process. Many investigators have determined the effect of varying CO_2 concentration, light intensity, temperature, and other factors upon the photosynthetic process, and important hypotheses and theories of the mechanism of photosynthesis have been derived from their observations. For obvious reasons, however, it has been difficult to determine the rôle of H_2O in the process, but now the use of D_2O suggests an approach to this important problem.

Preliminary reports from different laboratories show that photosynthesis is considerably reduced in heavy water. For example, it was found from direct measurements that with 99.9 per cent D_2O photosynthesis in the unicellular green alga *Chlorella* sp. was 0.41 of that with H_2O (8). This figure closely approximates the earlier estimate (based on chemical analyses of algae cultured in D_2O) that D is assimilated 41 per cent as rapidly as H (24).

Influence of D_2O on respiration.—The respiration of several organisms has also been studied and found to be markedly retarded in the higher concentrations of D_2O. In one series of experiments the oxygen consumption of luminous bacteria decreased rapidly as the concentration of D_2O increased from 0.02 to 86 per cent and extrapolation of the curve representing respiration as a function of D_2O concentration indicated zero respiration in 100 per cent heavy water (12). Other experiments showed that the oxygen consumption of yeast (32) and green algae (6) was reduced about one half in 100 per cent D_2O but that dilute D_2O had no influence on the respiration of wheat seedlings (7) or of yeast (32).

Influence of D_2O on muscles and nerves.—There has been much speculation concerning the effect of D_2O on nerve and muscle action, although few detailed experiments have been reported. It is clear, however, that some muscles at least are very sensitive to D_2O. Carefully controlled experiments (36) showed that the strength and frequency of the beat of isolated frogs' hearts were progressively diminished as the concentration of D_2O in Ringer's solution was increased from 0.02 to 99.2 per cent. The deleterious effect on hearts and other muscles was evident immediately, but D_2O seemed to have no influence on the nerves studied.

Mechanism of the action of D_2O on physiological processes.—The influence of D_2O on the rates of several physiological processes is well known, but the cause of the action is not clear.[3]

Evidence in the literature indicates that, with the possible exception of the membranes of mammalian erythrocytes (3, 21), cell membranes do not differ significantly in permeability to concentrated D_2O and to H_2O, and that the two liquids are not markedly different in ability to participate in establishing osmotic pressure (8, 17, 22).

Numerous chemical studies have shown that when sugars, cellulose, and many other organic and inorganic compounds are placed in contact with D_2O labile H atoms are quickly replaced by D atoms, and that the activity of the deuterium compound formed usually differs to a greater or lesser extent from that of the original compound (22). Similar exchanges probably occur

[3] It should be observed that the sensitiveness of different species of organisms to D_2O varies considerably and is not clearly correlated with their phylogenetic positions.

when living cells are immersed in D_2O (2). Thus the activities of different intracellular compounds, and hence the relative velocities of various reactions, may be altered when cells are exposed to D_2O. Since disturbance of the normal relative rates of physiological processes may cause serious injury to plants and animals, it seems possible that D_2O influences life processes by altering the established rate relations of mutually dependent intracellular reactions. Other chemical studies have shown that the ratio of the dissociated to the undissociated forms of several compounds changes when D_2O is substituted for H_2O as the solvent (16, 31). The ratio of dissociated to undissociated molecules in protoplasm might, therefore, be quite different in the two liquids. This kind of disturbance might seriously alter the relative rates of essential reactions in living cells and account for the observed effect of D_2O on living organisms.

D as an indicator in metabolism.—Although the initial and end products of many physiological processes are known, the intermediate steps by which one is converted into the other are, in many instances, unknown. Recent investigations indicate that the metabolic systems of mice and dogs are unable to distinguish some fatty acids hydrogenated with deuterium from those similarly treated with hydrogen. The analytical chemist can, however, detect the slight difference. Thus by feeding these compounds to animals and later analyzing their body fluids it is possible to trace the intermediate steps in different processes and to obtain elusive information about normal metabolism. For example, physiologists found that even when animals were fed a diet insufficient to maintain their weight, the fat in the diet was not available for use directly, but had first to be deposited in the fat depots (28), and in another experiment it was possible to trace several intermediate steps in the metabolism of the important substance cholesterol in dogs and to obtain information concerning its behavior in man.

Department of Botany
Columbia University
New York City

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